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IS 4467 (1996): Caramel [FAD 8: Food Additives]

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भारतीय मानक

कैरामल — विशिष्टि

(दूसरा पुनरीक्षण)

Indian Standard

CARAMEL — SPECIFICATION

(Second Revision)

ICS 67.220.20

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BUREAU OF INDIAN STANDARDS
MANAK BHAVAN, 9 BAHADUR SHAH ZAFAR MARG
NEW DELHI 110002

October 1996

Price Group 8

Food Additives Sectional Committee, FAD 8

FOREWORD

This Indian Standard (Second Revision) was adopted by the Bureau of Indian Standards, after the draft finalized by the Food Additives Sectional Committee had been approved by the Food and Agriculture Division Council.

The term caramel does not denote a single chemical substance. A wide range of different types of caramel are manufactured to suit the requirements of particular users. This standard, therefore, specifies limits for harmful impurities and such characteristics as will ensure an acceptable standard of quality. However, detailed specifications keeping in view the specific needs may be agreed to between the users and the manufacturers.

The standard was first issued in 1967. It was revised in 1980 and published in 3 parts, each part covering a different type of caramel, namely plain, ammonia process and ammonia sulphite process. Keeping in view the developments in trade practice and with a view to aligning it with International Standards, this standard is now being revised to cover caramel in one standard under the following 4 types.

Type I	Plain, caustic caramel;
Type II	Caustic sulphite caramel;
Type III	Ammonia process caramel; and
Type IV	Ammonia sulphite caramel.

In the preparation of this standard, due consideration has been given to the provisions under the *Prevention of Food Adulteration Rules*, 1955. However, this standard is subject to the restrictions imposed under these Rules, wherever applicable. These rules inter-alia prescribe

All food colours (including natural colouring matters and permitted synthetic food colours, their preparations or mixtures except saffron and carcumin) shall be sold only under the Bureau of Indian Standards Certification Mark'.

Caramel is a natural colouring matter.

Considerable assistance has been derived from the following publication in preparation of this standard:

> Specification for identity and purity of some food additives, FAO Nutrition Meeting Report Series No. 38. FAO/WHO, Rome, 1988.

For the purpose of deciding whether a particular requirement of this standard is complied with, the final value, observed or calculated, expressing the result of a test or analysis, shall be rounded off in accordance with IS 2 : 1960 'Rules for rounding off numerical values *(revised)*'. The number of significant places retained in the rounded off value should be the same as that of the specified value in this standard.

Indian Standard

CARAMEL — SPECIFICATION

(Second Revision)

1 SCOPE

1.1 This standard prescribes the requirements and methods of sampling and test for caramel for use in foodstuffs.

2 REFERENCES

The Indian Standards listed below contain provisions which through reference in this text, constitute provision of this standard. At the time of publication, the editions indicated were valid. All standards are subject to revision and parties to agreements based on this standard are encouraged to investigate the possibility of applying the most recent editions of the standards indicated.

IS No.	*Title*
1070 : 1992	Reagent grade water (*third revision*)
1699 : 1995	Methods of sampling and test for synthetic food colours (*second revision*)
2491 : 1972	Code for hygienic conditions for food processing units (*first revision*)

3 TERMINOLOGY

For the purpose of this standard, the following definitions shall apply.

3.1 Type Tests

Tests to prove conformity to the requirements of this standard. They are intended to approve the formulation and quality of the product at least in the beginning of marketing and/or certification. These tests are also conducted periodically to supplement the routine tests or whenever the basic formula or method is changed.

3.2 Routine Tests

Tests carried out on each lot to check the essential requirements that are likely to vary during production.

4 TYPES

4.1 Caramel colours are complex mixtures of compounds some of which are in the form of colloidal aggregates, manufactured by heating carbohydrates either alone or in the presence of foodgrade (extremely good quality) acids, alkalis, or salts including sodium and potassium salts. They are classified according to the reactants used in their manufacture as follows:

4.1.1 *Type I, Plain, Caustic Caramel*

It shall be prepared by heating carbohydrates with or without acids, alkalis or salts. No ammonium or sulphite compounds are used. It is mostly used in spirit industry.

4.1.2 *Type II, Caustic Sulphite Caramel*

It shall be prepared by heating carbohydrates with or without acids, alkalis or salts in the presence of sulphite compounds; no ammonium compounds are used.

4.1.3 *Type III, Ammonia Process Caramel*

It shall be prepared by heating carbohydrates with or without acids, alkalis or salts in the presence of ammonium compounds. No sulphite compounds are used. It is mostly used in beverage and beer industry.

4.1.4 *Type IV, Ammonia Sulphite Caramel*

It shall be prepared by heating carbohydrates with or without acids, alkalis or salts in the presence of both sulphite and ammonium compounds. It is mostly used in the soft drink industry.

5 RAW MATERIALS

5.1 The carbohydrate raw materials used are food-grade nutritive sweeteners consisting of sucrose, glucose, fructose, invert sugar, lactose, malt syrup, molasses, starch hydrolysates and fractions thereof and/or polymers thereof.

5.2 The acids used are food grade (extremely good quality) sulphuric acid, phosphoric acid, acetic acid, or citric acid and the alkalis used are food grade (extremely good quality) sodium, potassium or calcium hydroxide or mixtures thereof.

5.3 The salts used are food grade sodium or potassium carbonate, bicarbonate, phosphates (including dibasic phosphate and monobasic phosphate) and sulphates.

5.4 Where ammonium compounds are used, they shall be one or more of the following:

Ammonium hydroxide
Ammonium carbonate and bicarbonate
Ammonium phosphate
Ammonium sulphate
Ammonium sulphite, bisulphite and metabisulphite

5.5 Where sulphite compounds are used, they shall be one or more of the following:

Sulphurous acid
Potassium, sodium or ammonium sulphites or bisulphites.

5.6 Polyglycerol esters of fatty acids, corn oil or other food grade antifoams permitted under the PFA Rules may be used as antifoaming agents during manufacture.

6 REQUIREMENTS

6.1 Routine Tests

6.1.1 *Description*

Caramel shall be a dark brown to black liquid or solid material having the characteristic odour of burnt sugar and a pleasant, bitter taste. It shall be miscible with water. Its solutions, when spread in a thin layer on a glass plate should appear homogenous, transparent and have reddish-brown colour. Caramel shall be free from any other extraneous colouring matter.

6.1.2 The material shall also conform to the requirements given in Table 1. All requirements are on solid basis, except metallic impurities.

6.2 Type Test

6.2.1 The material shall also conform to the requirements given in Table 2. All requirements are on solid basis except metallic impurities.

6.3 For calculation on solid basis, refer Annex J.

6.4 The product shall be processed, packed, stored and distributed under hygienic conditions in licensed premises (*see* IS 2491 : 1972).

Table 1 Routine Test Requirements for Caramel
(*Clause* 6.1.2)

Sl No.	Characteristics	Requirements				Method of Test, Ref to	
		Type I Plain	Type II Caustic Sulphite	Type III Ammonia Process	Type IV Sulphite Ammonia	Annex of This Standard	Cl of IS 1699 : 1995
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
i)	Solid content, percent by mass	62 - 77	65 - 72	53 - 83	40 - 75	A	—
ii)	Colour intensity, percent by mass	0.01 - 0.12	0.06 - 0.10	0.08 - 0.36	0.10 - 0.60	B	—
iii)	Ammoniacal nitrogen, percent by mass, *Max* (*see* Notes 1 and 2)	0.01	0.01	0.4	0.5	C	—
iv)	4-Methylimidazole (4-MEI), mg/kg, *Max* (*see* Notes 1 and 2)	—	—	300 and 200 on an equivalent colour basis	1 000 and 250 on an equivalent colour basis	D	—
v)	Lead (as Pb), mg/kg, *Max*	5	5	5	5	—	15
vi)	Arsenic (as As), mg/kg, *Max*	3	3	3	3	—	15

NOTES

1 Requirement of ammonical nitrogen is based on a product having a minimum colour intensity prescribed at Sl No. ii); proportionately higher values for ammonical nitrogen apply for products of higher colour intensity.

2 For limits expressed on equivalent colour basis, *see* calculations given at Annex B.

Table 2 Type Test Requirements for Caramel
(*Clause* 6.2.1)

Sl No.	Characteristics	Requirements				Method of Test, Ref to	
		Type I Plain	Type II Caustic Sulphite	Type III Ammonia Process	Type IV Sulphite Ammonia	Annex of This Standard	Cl of IS 1699 : 1995
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
i)	Total sulphur, percent by mass	*Max*, 0.3	1.3 - 2.5	*Max*, 0.3	1.4 - 10.0	E	—
ii)	Sulphur dioxide (as SO_2), percent by mass, *Max*	—	0.2	—	0.5	F	—
iii)	Total nitrogen, percent by mass	*Max*, 0.1	*Max*, 0.2	1.3 - 6.8	0.5 - 7.5	G	—
iv)	Heavy metals, mg/kg, *Max*	25	25	25	25	—	16
v)	2-Acetyl-4-tetrahydroxy butylimidazole (THI), mg/kg, *Max* (*see* Notes 1 and 2 below Table 1)	—	—	40 and 25 on an equivalent colour basis	—	H	—
vi)	Mercury (as Hg), mg/kg, *Max*	0.1	0.1	0.1	0.1	—	15
vii)	Copper, mg/kg, *Max*	20	20	20	20	—	15

7 PACKING AND MARKING

7.1 Packing

The material shall be filled in amber-coloured glass or high density polyethylene containers or any other well-closed suitable containers with as little air space as possible. The containers shall be such as to preclude contamination of the contents with metals or other impurities.

7.2 Marking

Each container shall be legibly and indelibly marked with the following information:

a) Name and type of the material;
b) Source of manufacture;
c) Minimum net content in g or kg;
d) Batch or code number;
e) Date of manufacture;
f) Colour intensity (range); and
g) Any other requirements under the *Standards of Weights and Measures (Packaged Commodities) Rules*, 1977/*Prevention of Food Adulteration Rules*, 1955.

7.2.1 *BIS Certification Marking*

The product may also be marked with the Standard Mark.

7.2.1.1 The use of the Standard Mark is governed by the provisions of the *Bureau of Indian Standards Act*, 1986 and the Rules and Regulations made thereunder. The details of conditions under which the licence for the use of Standard Mark may be granted to manufacturers or producers may be obtained from the Bureau of Indian Standards.

8 SAMPLING

8.1 Representative samples for tests shall be drawn according to the method prescribed in 4 of IS 1699 : 1995.

9 QUALITY OF REAGENTS

Unless specified otherwise, pure chemicals and distilled water (*see* IS 1070 : 1992) shall be employed in tests.

NOTE — 'Pure chemicals' shall mean chemicals that do not contain impurities which affect the test method results of analysis.

ANNEX A

[*Table* 1, *Item* (i)]

DETERMINATION OF SOLID CONTENT

There are two methods for determining the solid content. Method I based on specific gravity determination can be used as a routine method. Method II shall be the referee method.

METHOD I — SPECIFIC GRAVITY METHOD

Pycnometer — A suitable capacity preferably a calibrated 100 ml volumetric flask with neck of 8 mm internal dia.

Procedure — Carefully clean pycnometer by filling with chromic acid cleaning solution and letting stand for a few hours. Empty pycnometer and rinse thoroughly with water. Fill with freshly boiled water previously cooled to about 15°C and place in a constant temperature bath at 20°C. After 30 min, adjust water level to graduated volume on pycnometer and stopper; remove from bath, wipe dry with clean cloth and weigh. Empty pycnometer, rinse several times with alcohol and then ether, let dry completely, remove ether vapour and weigh. Determine the weight of water at 20°C by subtracting weight of pycnometer from its weight when filled with H_2O.

Fill the dried pycnometer with sample, previously cooled to 15°C, using a long-stem funnel reaching below graduation mark, until level of the sample is upto lower end of neck of the flask. (Flow may be stopped by inserting glass rod of suitable size into funnel so as to close stem opening.)

Carefully remove funnel to prevent sample from coming in contact with neck. Place it in constant temperature bath at 20°C for 30 min, remove from the bath, wipe it dry and weigh flask and sample. Add water previously cooled and maintained to about 15°C almost to graduation mark, running it down-side of neck to prevent mixing with sample. Remove air bubbles if any by gentle tapping. Place flask in constant temperature bath at 20°C for 30 min. Dilute to volume at that temperature with water. Remove the flask from the bath, wipe it dry and weigh. From this deduct the weight of sample and calculate specific gravity. Obtain the corresponding percent solids and degree Baume from the Table 3.

Calculation:

Weight of water content of flask at 20°C	=	x
Weight of sample at 20°C	=	y
Weight of sample plus water at 20°C	=	z
Weight of water occupying space of sample	=	$x-(z-y)$
Specific gravity 20/20 of sample	=	$\dfrac{y}{x-(z-y)}$

METHOD II

The solid content of caramel colour is determined by drying a sample upon a carrier composed of pure quartz sand that passes a No. 40 but not a No. 60 sieve and has been prepared by digestion with hydrochloric acid, washed acid-free, dried and ignited. Mix 30.0 g of prepared sand accurately weighed with 1.5-2.0 g caramel colour accurately weighed and dry to constant weight at 60°C under reduced pressure 50 mm/Hg (6.7 kPa). Record the final weight of the sand plus caramel. Calculate the percent solids as follows:

$$\text{Percent solids} = \frac{w_f - w_s}{w_c} \times 100$$

where

w_f = final weight of sand plus caramel,
w_s = weight of sand, and
w_c = weight of caramel initially added.

Table 3 Degrees Brix, Specific Gravity, and Degrees Baume of Sugar Solutions (Plato Table)[1]

°Brix or % by Wt of Sucrose	Specific Gravity at		°Baume (Modulus 145)	°Brix or % by Wt of Sucrose	Specific Gravity at		°Baume (Modulus 145)
	20/20°	20/4°			20/20°	20/4°	
0.0	1.00000	0.998234	0.00	11.0	413	2288	.13
.2	078	9010	.11	.2	497	3121	.24
.4	155	9786	.22	.4	580	3954	.35
.6	233	1.000563	.34	.6	664	4788	.46
.8	311	1342	.45	.8	747	5625	.57
1.0	389	2120	.56	12.0	831	6462	.68
.2	467	2897	.67	.2	915	7300	.79
.4	545	3675	.79	.4	999	8140	.90
.6	623	4453	.90	.6	5084	8980	7.02
.8	701	5234	1.01	.8	168	9822	.13
2.0	779	6015	.12	13.0	252	50665	.24
.2	858	6796	.23	.2	337	1510	.35
.4	936	7580	.34	.4	422	2356	.46
.6	1015	8363	.46	.6	506	3202	.57
.8	093	9148	.57	.8	591	4050	.68
3.0	172	9934	.68	14.0	677	4900	.79
.2	251	10721	.79	.2	762	5751	.90
.4	330	1510	.90	.4	847	6602	8.01
.6	409	2298	2.02	.6	933	7455	.12
.8	488	3089	.13	.8	6018	8310	.23
4.0	567	3881	.24	15.0	104	9165	.34
.2	647	4673	.35	.2	190	60022	.45
.4	726	5467	.46	.4	276	0880	.56
.6	806	6261	.57	.6	362	1738	.67
.8	886	7058	.68	.8	448	2598	.78
5.0	965	7854	.79	16.0	534	3460	.89
.2	2045	8652	.91	.2	621	4324	9.00
.4	125	9451	3.02	.4	707	5188	.11
.6	206	20251	.13	.6	794	6054	.22
.8	286	1053	.24	.8	881	6921	.33
6.0	366	1855	.35	17.0	968	7789	.45
.2	447	2659	.46	.2	7055	8658	.56
.4	527	3463	.57	.4	142	9529	.67
.6	608	4270	.69	.6	229	70400	.78
.8	689	5077	.80	.8	317	1273	.89
7.0	770	5885	.91	18.0	1.07404	1.072147	10.00
.2	851	6694	4.02	.2	492	3023	.11
.4	932	7504	.13	.4	580	3900	.22
.6	3013	8316	.24	.6	668	4777	.33
.8	095	9128	.35	.8	756	5657	.44
8.0	176	9942	.46	19.0	844	6537	.55
.2	258	30757	.58	.2	932	7419	.66
.4	340	1573	.69	.4	8021	8302	.77
.6	422	2391	.80	.6	110	9187	.88
.8	504	3209	.91	.8	198	80072	.99
9.0	1.03586	1.034029	5.02	20.0	287	0959	11.10
.2	668	4850	.13	.2	376	1848	.21
.4	750	5671	.24	.4	465	2737	.32
.6	833	6794	.35	.6	554	3628	.43
.8	915	7318	.46	.8	644	4520	.54
10.0	998	8143	.57	21.0	733	5414	.65
.2	4081	8970	.68	.2	823	6309	.76
.4	164	9797	.80	.4	913	7205	.87
.6	247	40626	.91	.6	9003	8101	.98
.8	330	1456	6.02	.8	093	9000	12.09

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1) *Natl Bur. Std. Circ.* C440, pp. 614, 626 (1942). Based upon figures prepared by Kalserliche Normal-Eichungs-Kommission and accepted by International Commission for Uniform Methods of Sugar Analysis.

Table 3 — (*Continued*)

°Brix or % by Wt of Sucrose	Specific Gravity at		°Baume (Modulus 145)	°Brix or % by Wt of Sucrose	Specific Gravity at		°Baume (Modulus 145)
	20/20°	20/4°			20/20°	20/4°	
22.0	183	9900	.20	33.0	347	1453	18.19
.2	273	90802	.31	.2	445	2429	.30
.4	364	1704	.42	.4	543	3405	.41
.6	454	2607	.52	.6	641	4384	.52
.8	545	3513	.63	.8	739	5363	.63
23.0	636	4420	.74	34.0	837	6345	.73
.2	727	5328	.85	.2	936	7328	.84
.4	818	6236	.96	.4	5034	8313	.95
.6	909	7147	13.07	.6	133	9298	19.06
.8	.10000	8058	.18	.8	232	50286	.17
24.0	092	8971	.29	35.0	331	1275	.28
.2	183	9886	.40	.2	430	2265	.38
.4	275	.100802	.51	.4	530	3256	.49
.6	367	1718	.62	.6	629	4249	.60
.8	459	2637	.73	.8	729	5242	.71
25.0	551	3557	.84	36.0	1.15828	1.156238	19.81
.2	643	4478	.95	.2	928	7235	.92
.4	736	5400	14.06	.4	6028	8233	20.03
.6	828	6324	.17	.6	128	9233	.14
.8	921	7248	.28	.8	228	60233	.25
26.0	1014	8175	.39	37.0	329	1236	.35
.2	106	9103	.49	.2	430	2240	.46
.4	200	10033	.60	.4	530	3245	.57
.6	293	0963	.71	.6	631	4252	.68
.8	386	1895	.82	.8	732	5259	.78
27.0	1.11480	1.112828	14.93	38.0	833	6269	.89
.2	573	3763	15.04	.2	934	7281	21.00
.4	667	4697	.15	.4	7036	8293	.11
.6	761	5635	.26	.6	138	9307	.21
.8	855	6572	.37	.8	239	70322	.32
28.0	949	7512	.48	39.0	341	1340	.43
.2	2043	8453	.59	.2	443	2359	.54
.4	138	9395	.69	.4	545	3379	.64
.6	232	20339	.80	.6	648	4400	.75
.8	327	1284	.91	.8	750	5423	.86
29.0	422	2231	16.02	40.0	853	6447	.97
.2	517	3179	.13	.2	956	7473	22.07
.4	612	4128	.24	.4	8058	8501	.18
.6	707	5079	.35	.6	162	9527	.29
.8	802	6030	.46	.8	265	80560	.39
30.0	898	6984	.57	41.0	368	1592	.50
.2	993	7939	.67	.2	472	2625	.61
.4	389	8896	.78	.4	575	3660	.72
.6	185	9853	.89	.6	679	4696	.82
.8	281	30812	17.00	.8	783	5734	.93
31.0	378	1773	.11	42.0	887	6773	23.04
.2	474	2735	.22	.2	992	7814	.14
.4	570	3698	.33	.4	9096	8856	.25
.6	667	4663	.43	.6	201	9901	.36
.8	764	5628	.54	.8	305	90946	.46
32.0	861	6596	.65	43.0	410	1993	.57
.2	958	7565	.76	.2	515	3041	.68
.4	4055	8534	.87	.4	620	4090	.78
.6	152	9506	.98	.6	726	5141	.89
.8	250	40479	18.08	.8	831	6193	24.00

Table 3 — (*Continued*)

°Brix or % by Wt of Sucrose	Specific Gravity at		°Baume (Modulus 145)	°Brix or % by Wt of Sucrose	Specific Gravity at		°Baume (Modulus 145)
	20/20°	20/4°			20/20°	20/4°	
44.0	936	7247	.10	55.0	976	7535	.90
.2	20042	8303	.21	.2	6090	8674	30.00
.4	148	9360	.32	.4	204	9815	.11
.6	254	.200420	.42	.6	319	60955	.21
.8	360	1480	.53	.8	433	2099	.32
45.0	1.20467	1.202540	24.63	56.0	548	3243	.42
.2	573	3603	.74	.2	663	4390	.52
.4	680	4668	.85	.4	778	5537	.63
.6	787	5733	.95	.6	893	6686	.73
.8	894	6801	25.06	.8	7008	7837	.83
46.0	1001	7870	.17	57.0	123	8989	.94
.2	108	8940	.27	.2	239	70143	31.04
.4	215	10013	.38	.4	355	1299	.15
.6	323	1086	.48	.6	471	2455	.25
.8	431	2162	.59	.8	587	3614	.35
47.0	538	3238	.70	58.0	703	4774	.46
.2	646	4317	.80	.2	819	5936	.56
.4	755	5395	.91	.4	936	7098	.66
.6	863	6476	26.01	.6	8052	8262	.76
.8	971	7559	.12	.8	169	9428	.87
48.0	2080	8643	.23	59.0	286	80595	.97
.2	189	9729	.33	.2	404	1764	32.07
.4	298	20815	.44	.4	520	2935	.18
.6	406	1904	.54	.6	638	4107	.28
.8	516	2995	.65	.8	755	5281	.38
49.0	625	4086	.75	60.0	873	6456	.49
.2	735	5180	.86	.2	991	7633	.59
.4	844	6274	.96	.4	9109	8811	.69
.6	954	7371	27.07	.6	227	9991	.79
.8	3064	8469	.18	.8	346	91172	.90
50.0	174	9567	.28	61.0	464	2354	33.00
.2	284	30668	.39	.2	583	3539	.10
.4	395	1770	.49	.4	701	4725	.20
.6	506	2874	.60	.6	820	5911	.31
.8	616	3979	.70	.8	940	7100	.41
51.0	727	5085	.81	62.0	.30059	8291	33.51
.2	838	6194	.91	.2	178	9483	.61
.4	949	7303	28.02	.4	298	.300677	.72
.6	4060	8414	.12	.6	418	1871	.82
.8	172	9527	.23	.8	537	3068	.92
52.0	284	40641	.33	63.0	1.30657	1.304267	34.02
.2	395	1757	.44	.2	778	5467	.12
.4	507	2873	.54	.4	898	6669	.23
.6	619	3992	.65	.6	1019	7872	.33
.8	731	5113	.75	.8	139	9077	.43
53.0	844	6234	.86	64.0	260	10282	.53
.2	956	7358	.96	.2	381	1489	.63
.4	5069	8482	29.06	.4	502	2699	.74
.6	182	9609	.17	.6	623	3909	.84
.8	295	50737	.27	.8	745	5121	.94
54.0	1.25408	1.251866	29.38	65.0	866	6334	35.04
.2	521	2997	.48	.2	988	7549	.14
.4	635	4129	.59	.4	2110	8766	.24
.6	748	5264	.69	.6	232	9983	.34
.8	862	6400	.80	.8	354	21203	.45

Table 3 — (*Continued*)

°Brix or % by Wt of Sucrose	Specific Gravity at		°Baume (Modulus 145)	°Brix or % by Wt of Sucrose	Specific Gravity at		°Baume (Modulus 145)
	20/20°	20/4°			20/20°	20/4°	
66.0	476	2425	.55	77.0	442	1956	41.01
.2	599	3648	.65	.2	573	3263	.11
.4	722	4872	.75	.4	704	4571	.21
.6	844	6097	.85	.6	835	5881	.31
.8	967	7325	.95	.8	966	7192	.40
67.0	3090	8554	36.05	78.0	.40098	8505	.50
.2	214	9785	.15	.2	230	9819	.60
.4	337	31017	.25	.4	361	.401134	.70
.6	460	2250	.35	.6	493	2452	.79
.8	584	3485	.45	.8	625	3771	.89
68.0	708	4722	.55	79.0	758	5091	.99
.2	832	5961	.66	.2	890	6412	42.08
.4	957	7200	.76	.4	1023	7735	.18
.6	4081	8441	.86	.6	155	9061	.28
.8	205	9684	.96	.8	288	10387	.37
69.0	330	40928	37.06	80.0	421	1715	.47
.2	455	2174	.16	.2	554	3044	.57
.4	580	3421	.26	.4	688	4374	.66
.6	705	4671	.36	.6	821	5706	.76
.8	830	5922	.46	.8	955	7039	.85
70.0	956	7174	.56	81.0	1.42088	1.418374	42.95
.2	5081	8427	.66	.2	222	9711	43.05
.4	207	9682	.76	.4	356	21049	.14
.6	333	50939	.86	.6	490	2390	.24
.8	459	2197	.96	.8	625	3730	.33
71.0	585	3456	38.06	82.0	759	5072	.43
.2	711	4717	.16	.2	894	6416	.53
.4	838	5980	.26	.4	3029	7761	.62
.6	964	7245	.35	.6	164	9109	.72
.8	6091	8511	.45	.8	298	30457	.81
72.0	1.36218	1.359778	38.55	83.0	434	1807	.91
.2	346	61047	.65	.2	569	3158	44.00
.4	473	2317	.75	.4	705	4511	.10
.6	600	3590	.85	.6	841	5866	.19
.8	728	4864	.95	.8	976	7222	.29
73.0	856	6139	39.05	84.0	4112	8579	.38
.2	983	7415	.15	.2	249	9938	.48
.4	7111	8693	.25	.4	385	41299	.57
.6	240	9973	.35	.6	521	2661	.67
.8	368	71254	.44	.8	658	4024	.76
74.0	496	2536	.54	85.0	794	5388	.86
.2	625	3820	.64	.2	931	6754	.95
.4	754	5105	.74	.4	5068	8121	45.05
.6	883	6392	.84	.6	205	9491	.14
.8	8012	7680	.94	.8	343	50860	.24
75.0	141	8971	40.03	86.0	480	2232	.33
.2	270	80262	.13	.2	618	3605	.42
.4	400	1555	.23	.4	755	4980	.52
.6	530	2851	.33	.6	893	6357	.61
.8	660	4148	.43	.8	6031	7735	.71
76.0	790	5446	.53	87.0	170	9114	.80
.2	920	6745	.62	.2	308	60495	.89
.4	9050	8045	.72	.4	446	1877	.99
.6	180	9347	.82	.6	585	3260	46.08
.8	311	90651	.92	.8	724	4645	.17

Table 3 — (*Concluded*)

°Brix or % by Wt of Sucrose	Specific Gravity at		°Baume (Modulus 145)	°Brix or % by Wt of Sucrose	Specific Gravity at		°Baume (Modulus 145)
	20/20°	20/4°			20/20°	20/4°	
88.0	862	6032	.27	94.0	1096	8289	49.03
.2	7002	7420	.36	.2	239	9720	.12
.4	141	8810	.45	.4	382	11151	.22
.6	280	70200	.55	.6	526	2585	.31
.8	420	1592	.64	.8	670	4019	.40
89.0	559	2986	.73	95.0	1.50814	1.515455	49.49
.2	699	4381	.83	.2	958	6893	.58
.4	839	5779	.92	.4	2102	8332	.67
.6	979	7176	47.01	.6	246	9771	.76
.8	8119	8575	.11	.8	390	21212	.85
90.0	1.48259	1.479976	47.20	96.0	535	2656	.94
.2	400	81378	.29	.2	680	4100	50.03
.4	540	2782	.38	.4	824	5546	.12
.6	681	4187	.48	.6	969	6993	.21
.8	822	5593	.57	.8	3114	8441	.30
91.0	963	7002	.66	97.0	260	9891	.39
.2	9104	8411	.75	.2	405	31342	.48
.4	246	9823	.84	.4	551	2794	.57
.6	387	91234	.94	.6	696	4248	.66
.8	529	2647	48.03	.8	842	5704	.75
92.0	671	4063	.12	98.0	988	7161	.84
.2	812	5479	.21	.2	4134	8618	.93
.4	954	6897	.30	.4	280	40076	51.02
.6	.50097	8316	.40	.6	426	1536	.10
.8	239	9736	.49	.8	573	2998	.19
93.0	381	.501158	.58	99.0	719	4462	.28
.2	524	2582	.67	.2	866	5926	.37
.4	667	4006	.76	.4	5013	7392	.46
.6	810	5432	.85	.6	160	8861	.55
.8	952	6859	.94	.8	307	50329	.64
				100.0	454	1800	.73

ANNEX B

[*Table* 1, *Item* (ii)]

DETERMINATION OF COLOUR INTENSITY

B-0 Two methods have been specified. Any method may be used depending upon the facilities available.

B-1 EBC METHOD

B-1.1 Principle

A solution of the sample, of suitable known concentration, is compared visually with permanent colour standards. The colour intensity is expressed in arbitrary units, being calculated from the designation of the matching colour slide and the concentration of the solution.

B-1.2 Apparatus

B-1.2.1 *Colour Comparator*

Use any Lovibond — Tintometer type comparator.

B-1.2.2 *Colour Slides*

Use EBC glass colour slides which are composed of superimposed Lovibond red and yellow slides. The standard slide for the purpose of this determination is the 20° slide, although various other slides representing lower and higher intensities will be necessary in order to obtain a close colour match with the sample. The value of the 20° slide is equal to $4R + 15Y$ where R and Y are the values of the red and yellow slides, respectively, on the Lovibond Scale. The chromaticity co-ordinates of the 20° slide on the C.I.E. system are:

$$X = 0.497; Y = 0.443; Z = 0.060$$

B-1.2.3 *Glass Cell*

Use a 25-mm cell suitable for use with the comparator.

B-1.2.4 *Source of Artificial North Daylight*

Commission International de l 'Eclairage standard B (equivalent black body radiation temperature = 4 800 K) at an intensity of 100-1 000 lux and employing a white reflecting surface of over 95 percent reflectivity.

B-1.3 Procedure

Prepare a 0.1 percent *m/v* solution of the sample in freshly boiled and cooled distilled water. If the solution is not brilliant, clarify it by centrifuging, not by filtration. Place the solution in the cell, and compare it with the EBC colour slides. For the comparison, use either diffuse north daylight or the source of artificial north daylight specified above. (NOTE — Caramel solutions often leave a very apparent deposit on the sides of the glass cells, usually invisible, but capable of affecting the colour reading obtained. Remove the film by washing with a dilute solution of sodium carbonate or sodium hydroxide or by rubbing with cotton wool. Cells should be checked from time to time to ensure that, when filled with distilled water, they do not show any colour reading). When testing intensely coloured sample, it is necessary (in order to keep the colour intensity within the EBC scale) to use, for comparison, a solution less concentrated than 0.1 percent. In these cases, dilute the sample solution to a suitable known concentration.

B-1.4 Calculation

$$\text{EBC units} = \frac{S^\circ \times 0.1 \times 1\,000}{C}$$

where

S° = EBC colour slide which most nearly matches the intensity of the solution; and

C = concentration, percent *m/v*, of the solution tested.

Report results to two significant figures.

B-2 ABSORBANCE METHOD

B-2.1 Apparatus

B-2.1.1 *Spectrophotometer*

Equipped with a monochromator to provide bandwidth of 2 nm or less and of such quality that the stray-light characteristic is 0.5 percent or less. Suitable instruments are the Beckman DU, Beckman BD-G, Bausch and Lomb Spectronic 505, or other equivalent to these.

B-2.2 Procedure

Prepare a 0.1 percent *m/v* solution of the sample in freshly boiled and cooled distilled water. If the solution is not brilliant clarify it by centrifuging, not by filtering. Determine the absorbace of the clear or clarified solution in a 1-cm cell at 610 nm, with a suitable spectrophotometer previously standardized using water as the reference. Calculate the colour intensity of the caramel colour as follows:

$$\text{Colour intensity} = \frac{A_{610} \times 100}{\text{Percent solids}}$$

where percent solids is determined as described in Annex A.

B-3 CALCULATIONS ON AN EQUIVALENT COLOUR BASIS

Where additional limits for 4-MEI and THI are expressed on an equivalent colour basis the concentrations are first calculated on a solid basis as directed under Annex J, and then expressed on an equivalent colour basis according to the formula:

$$\text{Equivalent colour basis} = \frac{C_s}{\text{Colour intensity}} \times 0.1$$

where

Cs = concentration on a solids basis.

This gives content expressed in terms of a product having a colour intensity of 0.1 absorbance units.

ANNEX C

[*Table* 1, *Item* (iii)]

DETERMINATION OF AMMONIACAL NITROGEN

C-1 APPARATUS

C-1.1 Distillation Apparatus

Take a 500-ml receiving flask and connect it to a distillation apparatus consisting of a Kjeldahl connecting bulb and a condenser, in such a way that the condenser delivery tube is immersed beneath the surface of the acid solution in the receiving flask.

C-2 REAGENTS

C-2.1 Sulphuric Acid — 0.1 N.

C-2.2 Magnesium Oxide — Carbonate free.

C-2.3 Methyl Red Indicator Solution — 500 mg of methyl red in 100 ml of ethanol.

C-2.4 Sodium Hydroxide — 0.1 N

C-3 PROCEDURE

C-3.1 Add 25 ml of sulphuric acid to a 500-ml receiving flask and connect it to the distillation apparatus. Transfer about 20 g of the sample, accurately weighed, into a 800-ml long-neck Kjeldahl digestion flask, and to the flask add 2 g of magnesium oxide, 200 ml of water, and several boiling chips. Swirl the digestion flask to mix the contents, and quickly connect it to the distillation apparatus. Heat the digestion flask to boiling, and collect about 100 ml of distillate in the receiving flask. Wash the tip of the delivery tube with a few millilitres of water, collecting the washings in the receiving flask. Then add 4 to 5 drops of methyl red indicator and titrate with the sodium hydroxide, recording the volume (in ml) required as S. Conduct a blank determination and record volume (in ml) of the sodium hydroxide required as B.

C-4 CALCULATION

C-4.1 Calculate the percent of ammoniacal nitrogen in the sample by the formula $(S - B) \times 0.001\ 4 \times 100/M$, in which M is the mass of the sample taken in grams.

NOTE — In case of ammonia and ammonia sulphite caramels, 2 g sample shall be taken for the test.

ANNEX D

[*Table* 1, *Item* (iv)]

DETERMINATION OF 4-METHYLIMIDAZOLE

D-0 Two methods for determination of 4-methylimidazole have been given. Any of the two methods may be used, however in case of dispute Method II shall be used as a referee method.

D-1 METHOD I

D-1.0 Principle

D-1.0.1 Caramel colour is added to a basic celite column and eluted with mixture of chloroform and ethanol. The eluent is extracted with dilute sulphuric acid and the aqueous extract concentrated, brought to a known volume and neutralized to a slightly alkaline *p*H.

D-1.0.2 The alkaline extract obtained is analyzed by paper chromatography. The developed spots are visualized by spraying with diazosulfanilic acid reagent, and sodium carbonate solution separately. The intensity of the spots is compared with those of known standard. Then it is quantitatively estimated colorimetrically if found necessary by cutting the spot, eluting it in ethyl alcohol and determining the optical density of the solution at 505 mm in a suitable spectrophotometer or spectrocolorimeter. From the calibrated graph, prepared simultaneously, the amount of 4-MeI is read off.

D-1.0.3 It is assumed that no other components are present in the extract having the same Rf value as 4-MeI and forming a colour with diazosulfanilic acid reagent and sodium carbonate.

D-1.0.4 This method is sensitive for estimation of 4-MeI above 5 mg/kg in the caramel.

D-1.1 Apparatus

D-1.1.1 *Chromatography Column* — With stopcock, 25.0 mm × 250 mm.

D-1.1.2 *Chromatography Chamber* — 250 mm × 250 mm × 120 mm.

D-1.1.3 *Whatman No.* 1 *Chromatography Paper* — 225 mm × 270 mm.

D-1.1.4 *Vacuum Flask* — 100 ml.

D-1.1.5 *Constant Temperature Water-Bath*

D-1.1.6 *Glass Wool*

D-1.1.7 *Micropipette* — 10 μl capacity.

D-1.1.8 *Spraying Bottles*

D-1.1.9 *Air Drier*

D-1.1.10 *Separating Funnel* — 250 ml.

D-1.1.11 *Spectrophotometer or Spectrocolorimeter*

D-1.1.12 p*H Meter*

D-1.2 Reagents

D-1.2.1 *Celite* 545

D-1.2.2 *Sodium Hydroxide Solution* — 2 N.

D-1.2.3 *Sodium Carbonate Solution* — 20 percent *m*/*v*.

D-1.2.4 *Elution Solvent* — Chloroform, ethanol or methanol; 80 : 20, *v*/*v*.

D-1.2.5 *Sulphuric Acid Solution* — 0.05 N.

D-1.2.6 p*H Test Paper*

D-1.2.7 *Developing Solvent*

D-1.2.7.1 *System*(1) — Containing diethyl ether, chloroform, methyl alcohol and ammonium hydroxide, 80 : 20 : 20 : 4 (*v/v*).

D-1.2.7.2 *System*(2) — Containing 3 parts of *n*-propyl alcohol and 1 part of 0.2 N ammonia solution.

D-1.2.8 *Spraying Reagent No. I*

D-1.2.8.1 *Reagent* (a) — Prepare freshly a 5.0 percent (*m/m*) solution of sodium nitrite ($NaNO_2$), cool to 0°C.

D-1.2.8.2 *Reagent* (b) — Add 0.9 g of sulfanilic acid to 9.0 ml of concentrated hydrochloric acid (HCl, 32 percent *m/m*) taken in a 100 ml flask and dilute to 100 ml with distilled water. Cool to 0°C.

D-1.2.9 *Spraying Reagent No.II* — 5 percent (*m/v*) sodium carbonate solution.

D-1.2.10 *Stock Solution of 4-Mel* — Dissolve 100 mg of 4- methylimidazole in 100 ml of 0.1 N sulphuric acid. Store the solution in a refrigerator.

D-1.2.10.1 *Standard solution of 4-Mel* — Pipette out 1 ml of 4-Mel stock solution in a 10 ml flask and dilute to volume with distilled water. Bring the *p*H of the solution to 9 using solid sodium carbonate. The solution corresponds to 100 mg/kg. Similarly, prepare 150, 200, 250 and 300 mg/kg of standard 4-Mel.

D-1.3 Procedure

D-1.3.1 *Extraction*

D-1.3.1.1 Prepare a basic column packing by mixing well Celite 545 and 2 N sodium hydroxide solution in the proportion of 2 ml of 2 N sodium hydroxide to 3 g Celite 545. Place a fine glass wool plug at the base of the chromatographic column, followed by 5.0 g of basic column packing. Tap the packing firmly to a uniform mass.

D-1.3.1.2 Take 10.0 g sample of caramel in 150 ml beaker. To this add 6 g of 20 percent sodium carbonate solution. Mix well and add 12.0 g Celite 545. After thoroughly mixing add the mixture to the prepared column. Dry, wash the beaker with about 2.0 g Celite 545 and add this wash to column. Place a plug of glass wool at the top of the dry wash and tap the contents of the column.

D-1.3.1.3 Elute the column with elution mixture until 125 ml has been collected. The elution rate should be 5 ml/min and collected into 250 ml separating funnel. Extract the elute with 20.0 ml portion of 0.05 N sulphuric acid and then with second 10 ml portion of 0.05 N sulphuric acid. (This extract should be strongly acidic *p*H 3-by *p*H meter/test paper.) Transfer the combined aqueous layers to 100 ml vacuum flask and concentrate to 5 ml by drying under vacuum at a temperature not exceeding 55°C. Be sure that no bumping occurs and that the volume is not reduced below 4 ml.

D-1.3.1.4 Transfer this aqueous solution into a 10.0 ml volumetric flask, which also receives rinsings with approximately 1 ml portion of distilled water until the mark is reached. After mixing, transfer the aqueous concentrate to a suitable sample vial and treat with small portion of sodium carbonate anhydrous powder until carbon dioxide evolution ceases and *p*H meter/test paper indicates *p*H 9.

D-1.3.2 *Paper Chromatography*

D-1.3.2.1 Spot 10 μl of this solution on the chromatographic paper 3.0 cm from the bottom of the paper, and develop the chromatogram at 25°C to 28°C by ascending paper chromatography using either of the developing solvent. Allow the solvent to travel up to 15 cm.

D-1.3.2.2 Remove the paper from the tank and dry it by air-drier. Spray the paper with diazosulfanilic acid reagent until the paper is just wet and then develop the colour by a light spray of 5.0 percent *m/v* sodium carbonate (anhydrous) solution.

D-1.3.2.3 Estimate the amount of 4-methylimidazole in the caramel sample by comparison with the standard spots, obtained by spotting known concentration of 4-methylimidazole. The characteristic spots take bright maroon-purple colour.

D-1.3.3 *Colorimetic Determination of 4-Methylimidazole*

D-1.3.3.1 The quantitative estimation by colorimetry need not be carried out on a routine manner but shall be used as reference method in case of dispute.

D-1.3.3.2 Cut the maroon colour spots and elute in 20 percent ethyl alcohol (5-10 ml). Keep aside for 30 min and immediately note the absorbance of the solution at 505 nm. Side by side prepare the calibrated graph using various concentrations (mg/kg range) of 4-methylimidazole, and the amount of 4-methylimidazole in the unknown is then read off directly from the above calibrated graph.

D-2 METHOD II

D-2.0 Equipment

D-2.0.1 Pyrex glasswool, 22 mm × 300 mm Chormatography coloumn with PTFE stopcock (for example Kimax 17800).

D-2.0.2 *Polypropylene Beaker* — 150 ml (for example Nalge 1201).

D-2.0.3 *Round-Bottom Flask* — 250 ml (for example Pyrex 4320).

D-2.0.4 *Powder Funnel* — 75 mm.

D-2.0.5 *Spatula*

D-2.0.6 *Rotary Vacuum Evaporator*

D-2.0.7 *Hot Plate*

D-2.0.8 *Pan for Water Bath*

D-2.0.9 *Disposable Pasteur Pippets*

D-2.0.10 *Volumetric Flask* — 5 ml.

D-2.1 Reagents

D-2.1.1 The reagents should be ACS grade or equivalent where applicable.

D-2.1.2 *Acetone*

D-2.1.3 *Celite* 545

D-2.1.4 *Methylene Chloride*

D-2.1.5 *Sodium Hydroxide*

D-2.1.6 *Tetrahydrofuran*

D-2.2 Procedure

After thoroughly mixing the caramel colour sample by shaking or stirring, weigh a 10.00 g aliquot into a 150-ml polypropylene beaker. Polypropylene is considered superior to glass because of its hydrophobic surface which facilitates quantitative sample transfer. A 5.0 g portion of 3.0 N NaOH is added and thoroughly mixed to ensure that the *p*H of the entire sample exceeds 12. A 20 g portion of Celite 545 is added to the beaker, and the contents are mixed until a semi-dry mixture is obtained. This normally requires approximately 2 to 3 min. With samples of unusually high water content, the resultant caramel colour Celite 545 mixture may be overly wet. In such cases, a 5.00 g aliquot of caramel colour may be mixed with 2.5 g of 3.0 N NaOH and 15 g of Celite 545 and carried through the remainder of the analysis.

A plug of Pyrex glass wool is placed in the bottom of a 22 mm × 300 mm chromatographic column with PTFE stopcock. The caramel colour—Celite 545 mixture is placed in the column with the aid of a 75-mm powder funnel. The column contents are settled by repeatedly allowing the column to fall vertically about 10 cm to a padded surface. When properly settled, the caramel colour—Celite 545 mixture should occupy approximately the lower 250 mm of the column. Care should be exercised at this point to avoid a column bed which is either too loosely or too tightly packed. Loose packing will allow too rapid elution of the methylene chloride and possibly incomplete extraction. A too tightly packed column, for example the result of tapping down the column contents, can result in regions of the bed which are relatively inaccessible to the extraction solvent. This can also result in incomplete extraction solvent. With the stopcock open the column is filled with methylene chloride poured from the sample beaker. When the solvent reaches the glasswool plug, the stopcock is closed and the solvent is allowed to stand in contact with the bed for 5 min. The stopcock is then opened and the column is further eluted with methylene chloride until 200 ml have been collected in a 250 ml round-bottom flask. A 1.00 ml aliquot of 2 MEI internal standard solution (50.0 mg of 2 MEI/50.0 ml of methylene chloride) is added to the collected elute. The 2 MEI is well separted from the 4 MEI under the GLC conditions employed and has not been found in a caramel colour.

The bulk of the solvent is then removed from the eluate on a rotary vacuum evaporator operated at 45 - 50 kPa and with the round-bottom flask maintained at 35°C in a water-bath. The extracted residue is transferred quantitatively to a 5 ml volumetric flask with a disposable pasteur pipette, by rinsing the round-bottom flask several times with small (about 0.75 ml) portions of either tetrahydrofuran or acetone. Both solvents have been used with equal success. After mixing the contents throughly by several inversions of the flask, the extract is ready for GLC analysis. The extract should be analysed as soon as possible after their preparation, because stability problems have occasionally been encountered with extracts more than 1 day old.

The GLC analysis is carried out using a gas chromatograph equipped with a hydrogen flame detector. The column is glass, 1 mm × 6 mm o.d. filled with 7.5 percent Carbowax 20 M + 2 percent KOH on 90/100 mesh Anakrom ABS. The GLC parameters are as follows: carrier, nitrogen, 50 ml/min; hydrogen, 50 ml/min; oxygen 80 ml/min; injection port 200°C; column isothermal 180°C; detector 250°C; sample size 5 μl. All quantitation is done by using the internal standard technique.

ANNEX E

[*Table* 2, *Item* (i)]

DETERMINATION OF TOTAL SULPHUR

In the largest available casserole that fits in an electric muffle furnace, place 1-3 g MgO or equivalent quantity of $Mg(NO_3)_2 6H_2O$ (6.4 - 19.2 g), 1 g powdered sucrose, and 50 ml HNO_3. Add 5-10 g caramel colour. Place same quantities of reagents in another casserole for blank. Evaporate on steam bath to paste. Place casserole in cold electric muffle and gradually heat (525°C) until all NO_2 fumes are driven off. Cool, dissolve and neutralize with HCl (1+2.5), adding excess of 5 ml. Filter, heat to boiling, and add 5 ml 10 percent $BaCl_2 2H_2O$ solution dropwise. Evaporate to 100 ml, let stand overnight, filter, wash, ignite, and weigh the $BaSO_4$. Correct result for $BaSO_4$ obtained in blank and report as mg S/100 g. Commercial instruments that analyse for total sulphur such as, the Leco-Combustion/Titration procedure can also be used and are recommended for sample amounts of about 200 mg.

ANNEX F

[*Table* 2, *Item* (ii)]

DETERMINATION OF SULPHUR DIOXIDE

F-1 REAGNETS

F-1.1 Hydrogen Peroxide — 3 percent neutral solution (free from sulphate).

F-1.2 Barium Chloride — 10 percent (*m/v*) solution.

F-1.3 Hydrochloric Acid — 2 N.

F-1.4 Hydrochloric Acid, Concentrated — sp gr 1.18.

F-1.5 Carbon Dioxide Gas — From cylinder.

F-1.6 Sodium Hydroxide — 0.1 N solution.

F-1.7 Bromophenol Blue Indicator

Dissolve 0.5 g of bromophenol blue in 7.5 ml of 0.1 N sodium hydroxide and dilute to 1 000 ml.

F-2 APPARATUS

F-2.1 Assembly as shown in Fig. 1.

F-3 PROCEDURE

F-3.1 Measure 25 ml of the hydrogen peroxide into the conical flask and add 25 ml of distilled water. Measure 10 ml of hydrogen peroxide and add into the first Wohler tube (U-tube). In the second tube (U-tube serves as a guard) place 5 ml of a mixture of equal volumes of the hydrogen peroxide and barium chloride solutions, which has been slightly acidified with 2 N hydrochloric acid solution.

F-3.2 Introduce 500 ml of distilled water and 20 ml of concentrated hydrochloric acid into the round-bottom flask. Connect the carbon dioxide tube to the flask and boil the water for a short time in a current of carbon dioxide gas. Introduce about 20 g sample, accurately weighed, into the round-bottom flask, through the funnel shown in Fig. 1, continuing the flow of carbon dioxide gas. While adding the sample, see that there shall not be any back pressure. Boil it for 2 h (passing a slow current of carbondioxide gas throughout the test). Before the end of the distillation stop the flow of water through the condenser to allow any sulphur dioxide which is retained by the condensed moisture in the tube of the condenser to be driven over into the receiver.

F-3.3 As soon as the delivery tube joining the condenser is hot, disconnect the straight tube first, wash it with water. Combine the contents of first Wohler tube or (U-tube) in the receiver or conical flask. Cool the conical flask and titrate with the sodium hydroxide solution using the bromophenol blue indicator. Calculate the percentage of sulphur dioxide. One millilitre of 0.1 sodium hydroxide solution is equal to 3.203 mg of sulphur dioxide.

NOTE — The solution in the second U-tube should remain clear. If any precipitation of barium sulphate is formed, the test should be repeated with an increased volume of hydrogen peroxide present in the conical flask and first Wohler tube.

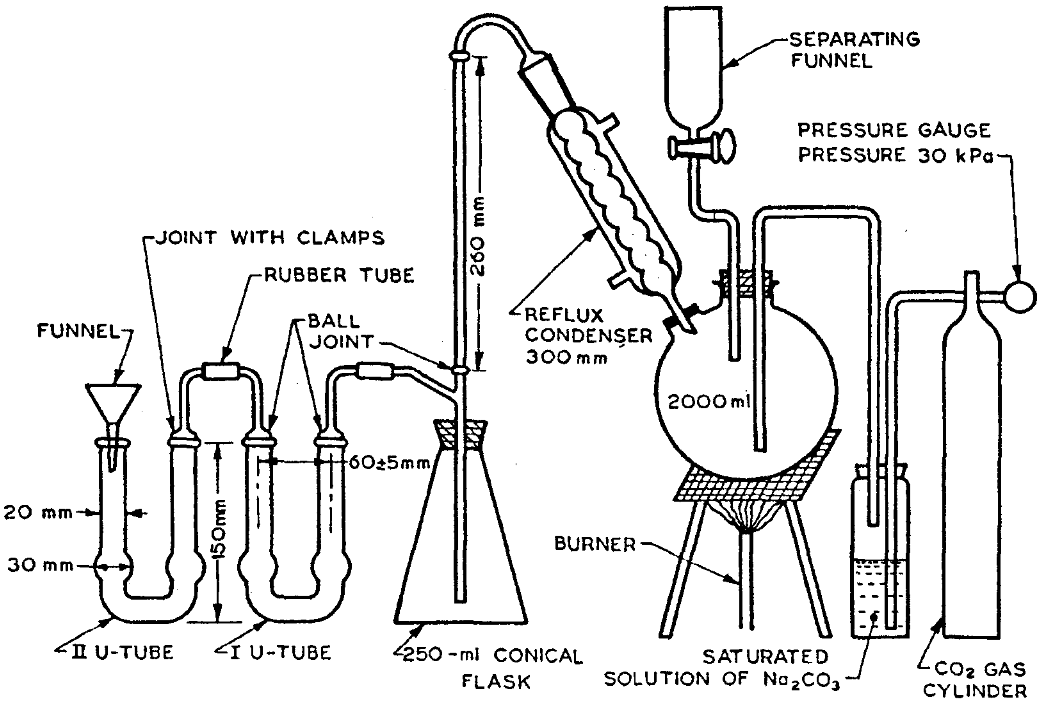

FIG. 1 ASSEMBLY FOR SULPHUR DIOXIDE

ANNEX G

[*Table* 2, *Item* (iii)]

DETERMINATION OF NITROGEN (KJELDAHL METHOD)

G-1 PROCEDURE

Transfer an accurately weighed or measured quantity of the sample equivalent to about 2 or 3 mg of nitrogen, to the digestion flask of a semi-micro Kjeldahl apparatus. Add 1 g of a powdered mixture of potassium sulphate and cupric sulphate (10 to 1), using a fine jet of water to wash down any material adhering to the neck of the flask, then pour 7 ml of sulphuric acid down the inside wall of the flask to rinse it. Add cautiously, down the inside of the flask, 1 ml of 30 percent hydrogen peroxide, swirling the flask during the addition (**Caution** — Do not add any peroxide during the digestion.) Heat over a free flame or an electric heater until the solution has attained a clear blue colour and the walls of the flask are free from carbonized material. Cautiously add 20 ml of water. Cool, then add through a funnel 30 ml of sodium hydroxide solution (2 in 5), and rinse the funnel with 10 ml of water. Connect the flask to a steam distillation apparatus and immediately begin the distillation with steam. Collect the distillate in 15 ml of boric acid solution (1 in 25) to which has been added 3 drops of methyl red/methylene blue solution and enough water to cover the end of the condensing tube. Continue passing the steam until 80 to 100 ml of distillate has been collected, then remove the absorption flask, rinse the end of the condenser tube with a small quantity of water, and titrate with 0.01 N sulphuric acid. Each ml of 0.01 N acid is equivalent to 0.140 mg (140 μg) of nitrogen.

Caution — Provide adequate ventilation in the laboratory and do not permit accumulation of exposed mercury.

NOTES

1 All reagents should be nitrogen-free, where available, or otherwise very low in nitrogen content.

2 When more than 2 to 3 mg of nitrogen is present in the measured quantity of the substance to be determined, 0.02 or 0.1 N sulphuric acid may be used in the titration if at least 15 ml of titrant is required. If the total dry weight of the material taken is greater than 100 mg, increase proportionately the quantitites of sulphuric acid and sodium hydroxide added before distillation.

ANNEX H

[*Table* 2, *Item* (v)]

DETERMINATION OF 2-ACETYL-4-TETRAHYDROXY BUTYLIMIDAZOLE (THI)

II-0 PRINCIPLE

THI is converted into its 2,4-dinitrophenylhydrazone (THI-DNPH). This derivatives is separated from excess reagent and carbonylic contaminants by HPLC on RP-8, then determined by its absorbance at 385 nm.

H-1 MATERIALS

2,4-Dinitrophenylhydrazine Hydrochloride Reagent

Commercial 2,4-dinitrophenylhydrazine (5 g) is added to concentrated hydrochloric acid (10 ml) in a 100-ml erlenmeyer flask and the latter is gently shaken until the free base (red) is converted to the hydrochloride (yellow). Ethanol (100 ml) is added and the mixture is heated on a steam bath until all the solid has dissolved.

After crystallization at room temperature the hydrochloride is filtered off washed with ether dried at room temperature and stored in a desiccator. On storge the hydrochloride is slowly converted to the free base. The latter can be removed by washing with dimethoxyethane. The reagent is prepared by mixing 0.5 g of 2,4-dinitrophenylhydrazine hydrochloride in 15 ml of 5 percent methanol in dimethoxyethane for 30 min. It should be stored in the refrigerator and be checked periodically by HPLC.

Cation-exchange resin (strong):
Dowex 50 AG × 8, H$^+$, 100-200 mesh.

Cation-exchange resin (weak):
Amberlite CG AG I, H$^+$, (100-200). Sediment two or three times prior to use).

Methanol Carbonyl-free: Methanol is prepared by treatment with Girard P reagent.

Dimethoxyethane. If impure, dimethoxyethane is purified by distillation from 2,4-dinitrophenylhydrazine in the presence of acid and redistilled from sodium hydroxide. Immediately prior to use it is passed through a column of neutral aluminium to remove peroxides.

Instrumental

Combination Column — The upper column (150 mm × 12.5 mm^2) filling height maximum 9 cm, or 200 mm × 10 mm, filling height maximum 14 cm with capillary outlet of 1 mm i.d.) is filled with weakly acidic cation-exchanger; bed height, approx 50 - 60 mm or 80 - 90 mm respectively. The lower column (total length 175 mm i.d. 10 mm, with capillary outlet and Teflon stopcock) is filled with strongly acidic cation-exchanger to a bed-height of 60 mm. As a solvent reservoir, a dropping funnel (100 ml) with Teflon stopcock is used. All parts are connected by standard ground-glass joints (14.5 mm).

HPLC — With the column specified above and an ultraviolet detector capable of reading at 385 nm.

Calibration

THI-DNPH is dissolved in absolute, carbonyl-free methanol (about 100 mg/l; calculated concentration of THI:47.58 ng/μl). A portion of this solution is diluted tenfold with methanol (4.7 ng THI/*μl*). THI-DNPH standard solution are stable for at least twenty weeks when stored in the refrigeraton.

II-2 PROCEDURE

Caramel colour (200-250 mg) is weighed accurately then dissolved in water (3 ml). The solution is transferred quantitatively to the upper part of a combination column. Elution with water is started and a total of about 100 ml of water is passed through the column.

The upper column is then disconnected. The lower column is eluted with 0.5 M HCl. The first 10.0 ml of elute are discarded then a volume of 35 ml is collected.

The solution is concentrated to dryness at 40°C and 15 torr. The syrup residue is dissolved in carbonyl-free methanol (250 μl) and the 2,4-dinitrophenylhydrazine reagent (250 μl) is added. The reaction mixture is transferred to a septum-capped vial and stored for 5 h at room temperature.

A volume of 5 μl (but also from 1 to 25 μl) is injected onto a LiChrosorb RP-8 (10 μm) HPLC column. The mobile phase is MeOH/0.1 M H_3PO_4 50/50 (*v/v*). Adjustment in mobile phase composition may be needed as column characteristics vary, depending upon the manufacturer. (Use of LiChrosorb RP-8, 10 μm, 250 mm × 4 mm "Vertex" column is recommended). At a mobile phase flow rate of 2 ml/min and column dimensions of 250 mm × 4.6 mm, THI-DNPH is eluted at about 6.3 ± 0.1 min. It is detected at 385 nm and the peak height is measured. The amount is calculated from a calibration curve prepared with THI-DNPH in methanol.

ANNEX J

(*Clauses* 6.3 *and* B-3)

CALCULATIONS ON A SOLIDS BASIS

The contents of total nitrogen, total sulphur, ammonical nitrogen, sulphur dioxide, 4-MEI and THI are expressed on a solid basis. The concentration (C_i) of each impurity is determined on an "as is" basis; the concentration (C_s) on a solid basis is then calculated using the formula:

$$C_s = \frac{C_i \times 100}{\text{Percent solids}}$$

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TO
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(Second Revision)

[*Page* 3, *Table* 2, *Sl No.* (i), *col* 4] — Substitute '*Max*, 2.5' *for* '1.3 – 2.5'.

[*Page* 3, *Table* 2, *Sl No.* (i), *col* 6] — Substitute '*Max*, 10.0' *for* '1.4 – 10.0'.

[*Page* 3, *clause* **7.2**(g)] — Insert the following at the end:

'h) Name and address of the manufacturer;
j) Instruction for storage like 'Store in cool and dark place away from sunlight'; and
k) Best before .... (Month & Year to be given by the manufacturer)'.

(FAD 8)

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